especially by the narrow specificity of the antiserum to hybrids. Similarly, though poly dG • poly dC was like DNA in its reactions with SLE serums, it does differ from DNA in its x-ray diffraction pattern (*11*), and its corresponding antiserum distinguishes it completely from DNA. This antiserum to poly dG • poly dC did not react with even the DNA of *Pseudomonas fluorescens*, which has a 72 percent content of dG • dC base pairs. Thus the high content of dG • dC base pairs does not appear to confer the immunospecific reactivity of poly dG • poly dC to that DNA molecule.

The patterns of cross-reactivity of these serums support the conclusion that immunochemical specificity depends on the conformations of the double helices rather than simply on reactions with a given base or the presence of either ribose or deoxyribose as the carbohydrate component.

Antibodies to denatured DNA can be readily induced in animals by several methods (*12*), but there are no unequivocal examples of experimentally induced antibodies having selective reactivity with native DNA. The only sources of such antibodies remain the serums of patients with SLE or serums of NZB/W mice which have a disease very like, or identical to, SLE (*13*).

From our experience it appears that there is a very specific tolerance, in the rabbit, to the native DNA conformation and perhaps to such molecules as poly dAT • poly dAT, which closely resemble DNA in structure (*11*). When other kinds of related polymers are used as immunogens, only those conformational features that are quite distinct from native DNA are recognized, as indicated by the finding that none of the antibodies to these double-helical molecules reacted with DNA. On the other hand, antibodies from patients with SLE do appear to recognize more general features of the DNA molecule that are also present in many synthetic polydeoxyribonucleotides; this may be the result of a breakdown of the usual tolerance. Because of the low degree or lack of cross-reactivity among the classes of polymer, some antiserums are useful as specific reagents for detecting either double-strand RNA or RNA-DNA hybrid molecules.

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References and Notes

1. Abbreviations used are: poly A, polyadenylate; poly U, polyuridylate; poly I, polyinosinate; poly C, polycytidylate; poly dA, polydeoxyadenylate; poly dT, polythymidylate; poly dG, polydeoxyguanylate; poly dC, polydeoxycytidylate; poly dAT, a single-strand polymer with alternating polydeoxyadenylate and polythymidylate. Double-strand copolymers are represented by the combination of two single strands separated by a center dot (for example, poly A · poly U). SLE, systemic lupus erythematosus; MBSA, methylated bovine serum albumin.
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Chemical Basis of Hashish Activity

Abstract. *A sample of hashish was extracted consecutively with petroleum ether, benzene, and methanol. When tested intravenously in monkeys only the petroleum-ether fraction was active. This material was further fractionated. The only active compound isolated was Δ^1-tetrahydrocannabinol. Cannabinol, cannabidiol, cannabichromene, cannabigerol, and cannabicyclol when administered together with Δ^1-tetrahydrocannabinol do not cause a change in the activity of the latter, under the experimental conditions used. These results provide evidence that, except for Δ^1-tetrahydrocannabinol, no other major, psychotomimetically active compounds are present in hashish.*

The active Δ^1-tetrahydrocannabinol (Δ^1THC) (*1*) and $\Delta^{1(6)}$THC (*2*) have been isolated from *Cannabis sativa* preparations. The former is considered the predominant active component (*3–5*). Both THC's have been found to reproduce *Cannabis* activity in humans (*6*) and animals (*7, 8*). However, a systematic fractionation of *Cannabis* samples monitored by biological testing has not yet been reported. Hence, doubt has been expressed (*9*) as to whether Δ^1THC and $\Delta^{1(6)}$THC are the only active constituents and can replace crude marihuana or its extracts in *Cannabis* research. This is a point of importance. The use of chemically undefined and variable materials such as marihuana or hashish or their extracts has inherent methodological disadvantages. Reproducible biochemical, pharmacological, and clinical experimentation could be considerably facilitated by the possible use of properly characterized compounds instead of crude mixtures.

We now report some observations which indicate that Δ^1THC is indeed the only major active constituent in hashish. $\Delta^{1(6)}$THC, if present, did not represent more than 1 percent of the amount of Δ^1THC in the sample that we investigated.

A sample of hashish (502 g) of Lebanese origin, about 18 months old, was extracted eight times with a total of 4 liters of petroleum ether (b.p. 60° to 80°C) at 22°C. The extract (162 g) contained 23.1 g of Δ^1THC as determined by gas-liquid chromatography (*10*). The residue was extracted twice with boiling benzene (total solvent volume, 1500 ml). The extract (22.5 g) contained no Δ^1THC (*10*) and was inactive (*11*). The residue remaining after the benzene treatment was extracted twice with 1 liter of boiling methanol. The extract (19.1 g) which contained no Δ^1THC (*10*) was also inactive (*11*).

The petroleum ether extract was administered in doses containing 250 μg and 500 μg of Δ^1THC per kilogram (as determined by gas-liquid chromatog-

raphy). In parallel experiments pure Δ^1THC in equivalent doses was administered under the same experimental conditions. The extracts as well as pure Δ^1THC were dissolved in polyethylene glycol 300 and then administered intravenously to adult rhesus monkeys of either sex. The behavior of the animals was followed for 1.5 hours before injection and for 5 hours afterward. Behavior was recorded as described (*7*) and was assessed by an observer who did not know the exact nature of the materials injected. At least two animals were used to test each dose. In all cases, changes observed included drowsiness, akinesia, ptosis, redness of conjunctivae, apathy, "tameness," and a characteristic crouched posture ["thinker position" (*7*)]. Extent and duration of the changes were qualitatively and quantitatively similar in all animals, who regained normal behavior within 3 hours of injection.

The petroleum ether fraction was further fractionated by preparative thin-layer chromatography (*12*). The polar material on or near the solvent application point was collected and extracted with chloroform. This fraction contains cannabinoid acids (*3*, *4*) and noncannabinoid materials. It was not active. All the material which on the thin-layer chromatography plate appeared between the above inactive polar fraction and cannabigerol, the most polar neutral cannabinoid, was collected, extracted with chloroform, and tested. It was also inactive. The rest of the material on the plate was extracted with chloroform and analyzed by gas-liquid and column chromatography (Florisil). The following compounds were identified (in order of increasing polarity): cannabicyclol (0.1 percent of the original hashish sample) (*13*), cannabidiol (5.3 percent) (*14*), Δ^1THC (4.6 percent) (*1*), cannabinol (0.8 percent) (*3*, *4*), cannabichromene (0.1 percent) (*15*), and cannabigerol (0.3 percent) (*16*). Except for Δ^1THC, none of these compounds was active. A number of minor unidentified peaks were observed on gas-liquid chromatography. However, none of these peaks represented more than 1 percent of the amount of Δ^1THC.

A series of experiments was performed to determine any possible behavioral effect due to interaction between the different components. Six animals (group A) were injected with a mixture of Δ^1THC, 250 μg/kg; cannabicyclol, 5.5 μg/kg; cannabidiol, 288 μg/kg; cannabinol, 43.5 μg/kg; cannabichromene, 5.5 μg/kg; cannabigerol, 16.5 μg/kg. Three monkeys (group B) were given a similar mixture, from which Δ^1THC had been excluded. Five animals (group C) received Δ^1THC (250 μg/kg) only. Solutions were made up in dimethylformamide and the volume injected was 0.05 ml/kg. In five animals of group A and in all of group C there were conspicuous and typical (*7*) behavior changes of similar extent and magnitude; these symptoms disappeared gradually within 3 hours. No observable abnormalities were noticed in monkeys of group B.

The results presented provide, in our view, strong evidence that, except for Δ^1THC, no other major active compounds were present in the analyzed sample of hashish. However, a number of inherent drawbacks in the above experiments should be pointed out: (i) The full parallelism between the activity in humans and monkeys is yet to be established, although it seems likely to be so at least as far as species sensitivity and major behavioral changes are concerned (*17*). (ii) The gross fractionation may have missed a minor active compound or a compound with low activity. (iii) In humans the usual self-administration is by smoking, while the above results were obtained by intravenous injection. Most of these drawbacks are, of course, common to and inevitable in almost any isolation and testing of active principles from natural products. With these reserves in mind, we nevertheless assume that any considerable variation from the activity of Δ^1THC would have been detected since, in all experiments, parallel tests with pure Δ^1THC were conducted.

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11. The term "inactive" indicates that after intravenous injection of doses up to 10 mg/kg, no behavioral changes were noticed. It should be emphasized that some fractions contain inactive Δ^1THC acids (*3*, *4*) which if heated (on evaporation of solvents, for example) may decarboxylate to yield active Δ^1THC. Therefore suitable precautions were taken in all manipulations.
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